

# Comparison of Bactericidal Activity of Serum Collected from Typhoid Patients and Normal Human Against *Salmonella typhi* at Various Incubation Time

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With an estimated 16-33 million cases of typhoid fever annually resulting in 500,000 to 600,000 deaths in endemic areas, the World Health Organization identifies typhoid, caused by the bacterium *Salmonella enterica* serovar *typhi* as a serious public health problem. Its incidence is the highest in children and young adults between 5 and 19 years old.

Susceptibility to the serum bactericidal system is a widespread characteristic of gram-negative bacteria. In addition to the many well-documented instances of enterobacterial susceptibility to complement, serum is known to possess bactericidal and bacteriolytic activity against susceptible representatives of practically every gram-negative genus so far examined.<sup>1</sup> The bactericidal effect of normal human serum plays an important role in host defense against bacterial infection. This phenomenon has been widely noted and studied since the late 1800s<sup>2</sup> and has been shown to be complement mediated.<sup>3,4</sup> Early colonization of the intestinal tract

by commensally bacteria ensures that small quantities of antibodies directed against the surface antigens of many types of gram-negative bacteria are present in the blood and tissue fluids of humans<sup>5</sup> and a variety of animals<sup>6</sup>. Thus, IgM and IgG antibodies directed against surface antigens of *E. coli*<sup>7</sup> and other enterobacteria<sup>8</sup> as well as against *Neisseria* species.<sup>7,9</sup> However, the development of the humoral immune response to O, H, and Vi antigens of *Salmonella typhi* has been regularly demonstrated during and after typhoid fever.<sup>10</sup> As a result, the sera of typhoid fever patients had been shown to harbor antibodies against O, H, and Vi antigens.<sup>11-13</sup> Therefore, sera from patient diagnosed with typhoid can provide the source for anti- *S. typhi* antibodies, along with the proteins of complement system.<sup>1</sup>

In the current study, bactericidal activity of *S. typhi* infected human serum against *S. typhi* was investigated. The requirement of complement pathways in serum mediated killing of *S. typhi* is also examined by means of inactivating both classical and alternative pathways of *S. typhi* infected human sera.

The *S. typhi* bacterial strain was collected from the Microbiology Department of Children's Hospital, Dhaka. The bacterial strain was collected as red colony with black centre on a Xylose Lysine

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Dechocolate (XLD) agar plate. The strain was tested using gram staining and serological studies. The organism was maintained at 4°C in XLD slant for not more than 15 days. Stock culture was streaked on a XLD plate and incubated at 37°C overnight. *S. typhi* from the overnight subculture was then transferred into 100 ml of normal saline, shaken to break the clumps and then adjusted to desired concentration by serial dilution.

Blood samples from nine healthy human with proper medical history were collected aseptically by venipuncture. Blood samples were kept in test tubes at 4°C overnight, centrifuged at 4000 rpm (Eppendorf AG 22331 Hamburg, Germany) for 10 min. The sera were separated and stored at minus 20°C in small aliquots. Sera from nine *S. typhi* infected patients who were diagnosed with typhoid fever were collected from Holy Family Red Crescent Hospital and Ibn Sina Diagnostic Centre. The sera were stored at minus 20°C in small aliquots.

The serum bactericidal assay for all nine *S. typhi* infected human sera and nine normal human sera were carried out according to Taylor and Kroll.<sup>14</sup> Bacterial cell suspension of 4.43 log<sub>10</sub>CFU/ml was treated with 40% human serum (this was found to be the optimum concentration) and incubated at 37°C for 15 min, 30 min, 45 min, 60 min and 90 min. The treated bacterial suspension was then spreaded on bismuth sulphite agar plate (stored at 4°C for 2 days before use) and the colonies were counted after overnight incubation. 60 µl bacterial cell suspensions (4.43 log<sub>10</sub>CFU/ml) was added to 40 µl physiological saline which was used as positive control. 60 µl physiological saline was added to 40 µl serum which was used as negative control. Role of complement pathways on the serum bactericidal activity was determined following the method described by several investigators.<sup>1</sup> Both classical and alternative pathways of nine *S. typhi* infected sera were inactivated by heating at 56°C for 30 min. After the heat treatment, the sera were subjected to serum bactericidal assay.

The mean values of the log<sub>10</sub>CFUs of *S. typhi* observed after serum bactericidal assay at various

incubation time in per ml of bacterial cell suspension treated with each of nine *S. typhi* infected human sera, nine normal human sera, and nine heat-treated *S. typhi* infected human sera were calculated and plotted against various incubation time (Figure 1).

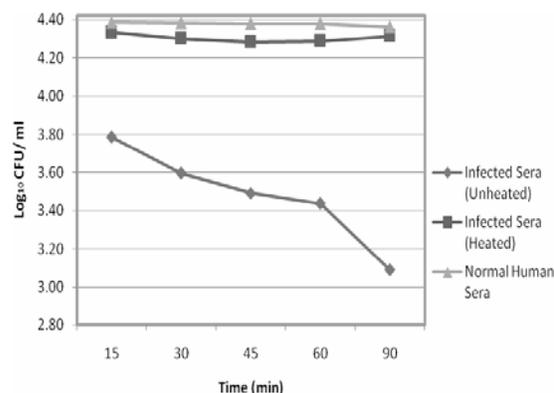


Figure 1. Mean bactericidal activity (log<sub>10</sub>CFU/ml) of *S. typhi* infected sera, normal human sera, and heat-treated *S. typhi* infected sera

The mean growth of *S. typhi* in the presence of infected human sera showed decline than the positive control starting from 3.79 log<sub>10</sub>CFU/ml after 15 min incubation time and 3.09 log<sub>10</sub>CFU/ml after 90 min incubation time as shown in Figure 1.

In the present study bactericidal activity of nine *S. typhi* infected human sera was examined against *S. typhi*. From this study it has been observed that *S. typhi* infected serum mediated killing increased with the increase of incubation time and showed maximum activity after 90 min incubation time (Figure 1).

The requirement for complement pathways in serum mediated killing of *S. typhi* was also examined by inactivating both classical and alternative pathways with heat treatment. Each of the nine *S. typhi* infected human serum was exposed to 56°C for 30 min according to Taylor<sup>1</sup> before mixing with the bacterial suspension. This treatment resulted in complete loss of serum bactericidal activity in most of the cases. This finding suggests the requirement of complement system pathways in serum mediated killing of *S. typhi*. Similar findings were observed in case of other organisms. There is a large body of

evidence indicating that the complement system is an important component of the host defense against infection with gram-negative bacteria.<sup>14</sup>

The requirement of the anti- *S. typhi* antibodies and the consequent activation of classical activation was investigated by examining the bactericidal activity of nine normal human sera, depriving of anti- *S. typhi* antibodies, against *S. typhi*. This study was unable to show any considerable killing of bacterial cell suspension, indicating the necessity of anti- *S. typhi* antibodies in the serum for classical pathway of activation and subsequent killing by Membrane Attack Complex (MAC) formation.

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